

2.1992-160 c2

CANADIANA

MAR 31 1992

THE PROJECT:
MONITORING OF OCCUPATIONAL EXPOSURE TO
**BIOLOGICAL MONITORING OF
OCCUPATIONAL EXPOSURE TO
ISOCYANATES**



A PROJECT FUNDED BY THE
OCCUPATIONAL HEALTH AND SAFETY
HERITAGE GRANT PROGRAM



TO: HERITAGE GRANT PROGRAM
ALBERTA OCCUPATIONAL HEALTH AND SAFETY

A REPORT ON THE PROJECT:

BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO ISOCYANATES

**BIOLOGICAL MONITORING OF
OCCUPATIONAL EXPOSURE TO
ISOCYANATES**

Submitted by

Sir C. Chen, Ph.D.
Laboratory Medicine
Foothills Hospital
1401 - 29th St. N.W.
Calgary, Alberta
T2N 2T5

July 1991



Digitized by the Internet Archive
in 2017 with funding from
University of Alberta Libraries

<https://archive.org/details/biologicalmonito00chan>

TO: HERITAGE GRANT PROGRAM
ALBERTA OCCUPATIONAL HEALTH AND SAFETY

A REPORT ON THE PROJECT:

BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO ISOCYANATES

a) Diisocyanates:

Submitted by

Siu C. Chan, Ph.D.
Laboratory Medicine
Foothills Hospital
1403 - 29th St. N.W.
Calgary, Alberta
T2N 2T9

July 1991

INTRODUCTION

A grant was provided by the Alberta Occupational Health and Safety Heritage Grant Program to study the feasibility of establishing a biological parameter to monitor occupational exposure of diisocyanates.

Diisocyanates are widely used in industry. Their major applications are in the production of flexible and rigid polyurethane foams, polyurethane paints, coatings, adhesives, sealants, and elastomers. They are hazardous substances. Local intense irritation occurs on direct skin contact, or accidental eye splashes. Acute vapour and aerosol exposure may cause respiratory symptoms such as chest tightness, shortness of breath, dry cough, and wheezing. Long term low level exposure may result in chronic dermatitis and asthma-like symptoms. In some cases, sensitization to diisocyanates may occur, and minimal re-exposure would trigger a major reaction.

The first objective of this project is to develop the capability of analyzing for diisocyanate-derived amines in urine. Then the urine samples collected in the 1987 health survey from autobody shop workers by Alberta Occupational Health and Safety will be analyzed, with the aim to correlate urinary level of diamines with clinical symptoms.

DEVELOPMENT OF ANALYTICAL CAPABILITIES

a) Diisocyanates:

A high performance liquid chromatographic (HPLC) method was developed to measure diisocyanates. The method is similar to that devised by Rastogi (1), which involves the conversion of the diisocyanates to urea derivatives by reacting them with 9-(methyl aminomethyl)-anthracene (MAMA). Detection is by absorbance measurement at 254 nm. The diisocyanates that are successfully analyzed by this procedure are 2,4- and 2,6-toluenediisocyanates (TDI), 1,6-hexane diisocyanate (HDI), 4,4'-methylenedi-p-phenyl diisocyanate (MDI), and isophorone diisocyanate (IPDI). The chemicals are measured in toluene solutions.

b) Diamines:

A gas chromatographic/mass spectrometric (GC/MS) method was developed to measure the diamines in urine. Diamines are either metabolites or reaction products of diisocyanates with water. As diisocyanates are very reactive compounds, they are not expected to be detectable in biological fluids, but rather the corresponding diamines would be found. The analytical method of Rosenberg and co-workers (2) is used with some modification. The method calls for the formation

of the heptafluorobutyryl derivatives of the diamines. The identity of the compound measured is confirmed by its full scan mass spectrum, while quantitative analysis is performed with the mass spectrometer in the selected ion monitoring (SIM) mode.

Prior to analysis, the diamines have to be extracted from the biological matrix (urine). This is achieved by first alkalinizing the matrix with saturated sodium hydroxide, and then followed by extraction with toluene.

METABOLIC STUDIES

In order to verify that when exposed to 2,4-TDI, 2,6-TDI, HDI, MDI, and IPDI, the corresponding diamines, 2,4-TDA, 2,6-TDA, HDA, MDA, and IPDA could be measured in the urine, metabolic studies involving rats were carried out.

Male Sprague-Dawley rats weighing 200 to 250 g were used for the experiments. They were acclimated for a week with free access to food (Teklad Rat Chow) and water in a temperature and humidity controlled room with a 14-h light cycle. Prior to exposure to the different diisocyanates, a rat was shaved on the abdomen in an area approximately 6x10 cm. A solution was prepared by dissolving 1 mg of the diisocyanate in 1 mL of toluene. 100 μ L of the solution were applied to the shaved area. After exposure, the rat was placed in a metabolic cage with free access to water, and urine were collected at different times for 24 hours.

It has been shown in the literature that these diamines are metabolized in the liver through conjugation to form acetylated compounds (3,4), therefore analysis of urine samples from these metabolic studies is divided into two parts. Analysis of free (unconjugated) diamines was performed after extraction of the diamines from alkalinized urine into toluene. For measurement of total (free and conjugated) diamines, the urine was first subjected to acid hydrolysis by boiling with 6M hydrochloric acid. The urine was then alkalinized, and the diamines were extracted into toluene.

In these studies, it was confirmed that the excretion products of 2,4-TDI, 2,6-TDI, HDI, and MDI are their corresponding diamines, as previously reported in the literature (2,3,4,5). The analyses were performed by GC/MS with the mass spectrometer operated in the full scan mode. The mass spectrum of the metabolite was compared to that of a commercially available standard. No other metabolites were detected in these studies.

IPDI is a newer diisocyanate that is being used in the manufacture of polyurethane paint. No metabolic study has

been published in the literature. HPLC analysis showed the commercially available IPDI consists of two isomers. Excretion study in rats also demonstrated the presence of two isomeric metabolites. These metabolites are identified as isomeric IPDA through mass spectral interpretation. Further evidence was provided by results from chemical ionization/MS analysis of these compounds. However, as there is no commercial source of IPDA, this is the extent we can support the identity of these metabolites.

Quantitative analyses were performed with the mass spectrometer operating in the SIM mode. Our study showed the majority of the diamines are excreted in the conjugated forms, a smaller, though significant, portion is excreted free. The ratios between the total diamine concentration to that of the free diamine ranged between 8 to almost 80. The diisocyanates are absorbed rapidly through the skin, metabolized, and excreted in rats. For all the rats we have studied, more than 50% of the administered dose was excreted in the urine within 24 hours, assuming the dose was completely absorbed.

URINE SAMPLES FROM AUTOBODY SHOP WORKERS

A total of one hundred and fourteen (114) frozen urine samples were sent to our laboratory for analysis. These samples had been under custody of the Laboratory Services Section, Alberta Occupational Health and Safety. Eighty nine (89) of these were collected in the 1987 health survey on workers from autobody shops in Alberta, and the remaining twenty five (25) were collected since the survey. These samples were received on September 5, 1990.

All analyses were made after acid hydrolysis of the urine sample, or in other words, the amounts of diamines reflect the sum of the free and conjugated moieties. The samples were first simultaneously screened for 2,4- and 2,6-TDA, HDA, IPDA, and MDA by GC/MS in the SIM mode. Presumptive positives are subjected to confirmation and quantification. Of these 114 samples, only 3 samples were shown to be positive: 2 in TDAs and 1 in HDA.

Sample 2025 was tested positive for 2,4- and 2,6-TDA. The worker complained of depression without any particular reason.

Sample 2060 was also tested positive for 2,4- and 2,6-TDA. The worker demonstrated many symptoms. He had problems with concentration, comprehension, and short term memory. He felt irritated and depressed without any particular reason. He was abnormally tired, and experienced palpitation of the heart, sweating, and tightness of the chest.

Sample 2067 was tested positive for HDA. This worker felt he had a short memory.

CONCLUSION

We have developed the technical capability of measuring urinary level of diamines. Our work confirmed previous finding that HDI, MDI, and TDI are excreted as their diamines. We had also provided evidence, for the first time, that IPDI is also excreted as the corresponding diamine, IPDA. The majority of these diamines are excreted in the conjugated form. A paper describing the technical details of the methodology will be published in a peer-reviewed journal.

Whether urinary level of diamines can be used as a parameter for biological monitoring of occupational exposure remains inclusive. Only 3 out of the 115 samples proved to be positive. Many workers with severe clinical symptoms were tested negative. The lack of correlation could be explained as follows:

1. The urine samples may have deteriorated due to long period of storage (over 3 years).
2. As the 1987 survey was geared towards the health effects of diisocyanates exposure, rather than the viability of biological monitoring, the conditions of urine collection may not be optimal.
3. Non-existence of any correlation.

More work needs to be done to answer the question whether biological monitoring is an useful technique to gauge the occupational exposure to diisocyanates.

RECOMMENDATION

As discussed in the above section, it will be very useful to organize another health survey with the aim of testing the feasibility of biological monitoring of exposure to diisocyanates in the workplace. This would be an interdisciplinary undertaking involving occupational physicians, industrial hygienists, and toxicologists. We will be interested in participating in such a project.

SIDELIGHT

The safety of the polyurethane-covered breast implants has been called into question recently. There have been intense public debate in the late 1990, and early 1991. The regulatory authorities, both in Canada and the United States,

are very concerned about the situation. Public pressure resulted in the world-wide recall of these products by the manufacturer in April, 1991. Because of our expertise in analyzing urinary diamines, we were able to make a contribution to this debate. We were first in providing evidence that 2,4-TDA, a suspected carcinogen, is present in the urine of a woman with these implants. A copy of our paper, as well as the news release about it, are enclosed for reference.

REFERENCES

1. Rastogi, S.C. Analysis of diisocyanate monomers in chemical products containing polyurethanes by high pressure liquid chromatography. *Chromatographia* 28: 15-8 (1989).
2. Rosenberg, C. and Savolainen, H. Determination in urine of diisocyanate-derived amines from occupational exposure by gas chromatography-mass fragmentography. *Analyst* 111: 1069-71 (1986).
3. Brorson, T., Skarping, G., Sandstrom, J.F., and Stenberg, M. Biological monitoring of isocyanates and related amines. I. Determination of 1,6-hexamethylene diamine (HDA) in hydrolysed human urine after oral administration of HDA. *Int Arch Occup Environ Health* 62: 79-84 (1990).
4. Cocker, J., Gristwood, W., Wilson, H.K. Assessment of occupational exposure to 4,4'-diaminodiphenylmethane (methylene dianiline) by gas chromatography-mass spectrometry analysis of urine. *Brit J Ind Med* 43: 620-5 (1986).
5. Rosenberg, C., and Savolainen, H. Determination of occupational exposure to toluenediisocyanate by biological monitoring. *J Chromatogr* 367: 385-92 (1986).

Detection of Toluenediamines in the Urine of a Patient with Polyurethane-Covered Breast Implants

Siu C. Chan,¹ Dale C. Birdsell,² and Claire Y. Gradeen¹

Breast prostheses are implanted for augmentation or during reconstructive surgery. One of the more commonly used prostheses is the polyurethane-sponge-covered silicone gel implant. Some clinicians are concerned about the safety of this product because the polyurethane foam disintegrates in vivo, and its subsequent fate is not known. Polyurethane is a polymer formed by reacting diisocyanates and polyols. This study indicates that the polymer sponge breaks down into its reactive monomers, 2,4- and 2,6-toluenediisocyanate, which are converted into their corresponding diamines. We present evidence of the excretion of the diamine metabolites in the urine of a patient implanted with polyurethane-covered prostheses.

Additional Keyphrases: *toxicology · gas chromatography-mass spectrometry*

The polyurethane-sponge-covered silicone gel breast implant was developed to overcome one of the main problems with the smooth-walled silicone breast prosthesis used for breast augmentation or reconstruction after ablative surgery: encapsulation by fibrous scar tissue, which may contract and compress the implant (1). This compression deforms the implant into a sphere and makes it undesirably firm. The cause of this scar encapsulation is thought to be organization of the periprosthetic blood clot, subclinical infection, excessive foreign-body reaction, or a combination of these factors.

The addition of a thin covering of polyurethane sponge to the surface of the smooth-walled silicone gel implant prevents the development of scar encapsulation that leads to spherical deformity and firmness. The scar that develops in its place is so enmeshed in the open-pore sponge that the contraction of the scar is multidirectional, diminishing or eliminating the spherical deformation and the compression of the implant (2).

The polyurethane-sponge-covered implants are not without complications. Infections, allergic reactions, and malpositions can occur. Occasionally, surgeons must reopen the breast wound and remove the implant. Also, the polyurethane sponge separates and disintegrates from the implant (3).

These implants are controversial because no one knows exactly what happens to the polyurethane sponge

and whether the breakdown products from the sponge have any adverse effects on the body.

Polyurethane is a polymer formed by reacting polyols and diisocyanates. The reactant most commonly used is toluenediisocyanate (TDI).³ TDI is a reactive compound and is converted into toluenediamine (TDA) on contact with water. TDI is a known irritant to the eyes, upper respiratory tract, and skin (4), and TDA has been shown to be a mutagen and to produce liver cancers in rats (5). Although the foam coating is known to disintegrate in the body, not much is known about its fate. However, if polyurethane breaks down into its monomers, then TDA would be expected to show up in the body. Indeed, we report here the presence of TDA in body fluids from a patient who has polyurethane-covered implants.

Case History

A 41-year-old woman underwent aesthetic breast augmentation in October 1988. Silicone gel implants were placed below the pectoral muscles. Despite this submuscular position, results were undesirable because of encapsulation of the left implant and lateral displacement of the right implant. In November 1989, these submuscular, smooth-walled implants were removed and a polyurethane-sponge-covered implant (Meme[®]; Surgitek, Racine, WI 53404) was placed in a new pocket created between the breast and the pectoral muscles on each side. Over the next several weeks, the implant on the left gradually became encapsulated and firm, and this side appeared to grow larger. On June 1, 1990, the left breast was re-explored and the implant was found to be encapsulated. The capsule was entered, and the gel implant was easily extracted. The surface of this gel implant was rough, indicating that the gel wall either had been designed to be rough or was coated with an adhesive. The polyurethane cover appeared partially absorbed, and it was enmeshed in, or integrated with, the scar encapsulating the implant. This scar was completely removed, revealing that the apparent enlargement of the left side was the result of a collection of fluid in the submuscular pocket where the previous implant had been placed. The submuscular smooth-walled scar was drained and partially excised. A new Meme implant was inserted.

Departments of ¹ Laboratory Medicine and ² Surgery, Foothills Hospital, 1403 29th St. N.W., Calgary, Alberta, Canada T2N 2T9. Received December 26, 1990; accepted March 18, 1991.

³ Nonstandard abbreviations: TDI, toluenediisocyanate; TDA, toluenediamine; MDA, methylenediphenyldiamine; and HFB, heptafluorobutylryl.

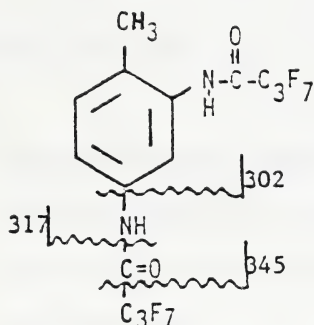


Fig. 2. Molecular structure of the heptafluorobutyl derivative of 2,4-toluenediamine (514 Da) and the interpretation of the major ions, $m/z = 302$, $m/z = 317$, $m/z = 345$, and $m/z = 514$ (the molecular ion)

Table 1. Concentration of 2,4- and 2,6-Toluenediamines in Samples Collected from a Patient with Polyurethane-Coated Breast Implants

Date of collection	Creatinine, mmol/L	TDA concn, $\mu\text{g/L}$		
		Free TDAs	Bound	
			2,4-TDA	2,6-TDA
<i>Urine</i>				
06/01/90	1.0	n.d.	0.27	0.15
06/21/90	3.8	n.d.	0.82	0.26
07/06/90	8.2	n.d.	1.69	0.61
07/19/90	2.8	n.d.	0.42	0.17
08/02/90	3.0	n.d.	0.55	0.25
08/20/90	1.2	n.d.	0.25	0.11
<i>Tissue</i>				
			Concn, $\mu\text{g/g}$	
06/01/90	—	n.d.	27.22	6.02
n.d., not detected.				

all negative for TDA.

The results of this study indicate that the polyurethane cover in the breast implant had disintegrated in vivo. Our evidence is the presence of the disintegration products, TDAs, in urine from the patient. The two possible sources of the TDAs cannot be differentiated by the chemical analyses performed. The first possibility, that polyurethane fragments or their breakdown polymers are excreted in the urine, is not very probable, because the fragments and polymers are large molecules and would not be easily absorbed, circulated, and excreted. The more likely explanation is that the polymer is broken down in vivo into its monomeric components, and the resulting TDIs are chemically converted into TDAs by reaction with body water and absorbed. The TDAs are in turn acetylated and excreted in the urine. Indeed, polyurethane can be degraded into the

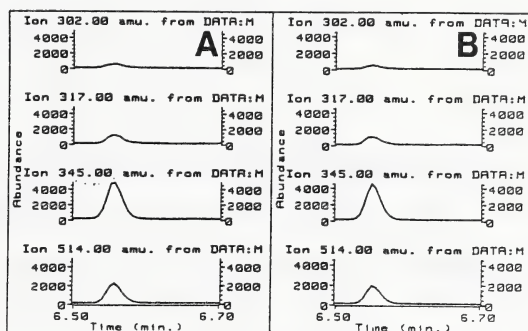


Fig. 3. Ion chromatograms of the heptafluorobutyl derivative of 2,4-toluenediamine from a hydrolyzed urine sample (A) and those from a 5 $\mu\text{g/L}$ standard solution (B)

corresponding diamine in vitro by a proteolytic enzyme (8).

The next step for our research will be to identify TDAs in the blood or acetylated TDAs in the urine of the patient. This would unequivocally prove degradation of the polymer into its monomers. In any case, we report the detection in urine of material that can be traced to the polyurethane-covered breast implants.

The development of the analytical techniques for these studies was supported by a grant from the Heritage Grant Program of the Alberta Department of Occupational Health and Safety.

References

1. Brandt B, Breiting V, Christensen L, Neilsen M, Thomsen JL. Five years experience of breast augmentation using silicone gel prostheses with emphasis on capsule shrinkage. *Scand J Plast Reconstr Surg* 1984;18:311-6.
2. Hester TR, Nahai F, Bostwick J, Cubic J. A 5-year experience with polyurethane-covered mammary prostheses for treatment of capsular contracture, primary augmentation mammoplasty, and breast reconstruction. *Clin Plast Surg* 1988;15:569-85.
3. Slade CL, Peterson HD. Disappearance of the polyurethane cover of the Ashley Natural Y prosthesis. *Plast Reconstr Surg* 1982;70:379-82.
4. Schnorr T, Egilman D. Toluene diisocyanate and other isocyanates. In: Zenz C, ed. *Occupational medicine, principles and practical applications*, 2nd ed. Chicago: Year Book Medical Publishers, 1988:1016-21.
5. Ito N, Hiasa Y, Konishi Y, Marugami M. The development of carcinoma in liver of rats treated with *m*-toluenediamine and the synergistic and antagonistic effects with other chemicals. *Cancer Res* 1969;29:1137-45.
6. Cocker C, Gristwood W, Wilson HK. Assessment of occupational exposure to 4,4'-diaminodiphenylmethane (methylenedianiline) by gas chromatography-mass spectrometry analysis of urine. *Br J Ind Med* 1986;43:620-5.
7. Rosenberg C, Savolainen H. Determination of occupational exposure to toluene diisocyanate by biological monitoring. *J Chromatogr* 1986;367:385-92.
8. Marchant RE, Zhao Q, Anderson JM, Hiltner A. Degradation of a poly(ether urethane urea) elastomer: infra-red and XPS studies. *Polymer* 1987;28:2032-9.

caused concern among clinicians because polyurethane foam disintegrates in the body, and it is unclear whether the breakdown products from the sponge have any adverse effects on the body.

The new study reports that the patient's body fluids contained breakdown products of polyurethane. The laboratory method detected toluenediamines (TDA) in urine and tissues; TDAs have been reported to produce liver cancer in rats. The TDAs that were found either were formed in the patient's body or were produced from related breakdown products in the patient samples. No TDAs were found in urine samples from other women. The authors conclude that the polyurethane covering was degraded in the body to the carcinogen or to closely related compounds that can be converted to TDA.

While the study indicates that the polyurethane cover of the implant breaks down into 2,4-TDA, the researchers caution that more research is needed to demonstrate definitively that this occurs in the patient's body.

(Enclosed is a preprint of the Clinical Chemistry article.)

Clinical Chemistry -- consistently ranked at the top in overall impact on the scientific world -- contains original, peer-reviewed scientific works including case histories, method texts, research findings, technological discoveries and book reviews.

page three of three

The journal is published monthly by the American Association for Clinical Chemistry, Inc. (AACC) and has a circulation of more than 20,000.

AACC is a Washington, DC-based nonprofit professional association with a membership of nearly 10,000 clinical chemists, pathologists, medical technologists and others in related fields. Through educational services and publications, AACC works to improve and advance laboratory services to enhance public health and patient care.

#

Prepared by : Claire Gradeen BSc., R.T.

Reviewed: 91-09-17

Revised: 91-10-31

Professional Approval: _____

1.0 DETERMINATION OF DIISOCYANATE METABOLITES
IN BIOLOGICAL FLUID
HEWLETT-PACKARD 5890 GAS CHROMATOGRAPH/
5970 MASS SPECTRAL DETECTOR

2.0 PRINCIPLE

Several investigators have reported the urinary excretion of conjugated diamines derived from monomeric diisocyanates in hydrolysed urine (2-4). The conjugates are likely to be acetylated since primary aliphatic and aromatic amines are known to be readily acetylated in vivo (5).

Deconjugation of bound diamines is effected by strong hydrolysis to free all the diamines from conjugates. The freed diamines are determined after alkaline extraction in toluene.

Since the diamines are volatile and decompose in the GC system, they are derivatized with heptafluorobutyril anhydride (HFBA) prior to analysis. The derivatized extracts are then injected into a gas chromatograph/mass selective detector (GC/MSD), operating in the SIM mode.

3.0 SPECIMEN REQUIREMENTS

- 3.1 The urine samples should be spun down to remove any particulate matter, and frozen at -20°C.
- 3.2 The creatinine levels should be determined to correct for urine concentration.

4.0 REAGENTS, SUPPLIES AND EQUIPMENT

4.1 REAGENTS

4.1.1 Diamine stock standards (1mg/mL)

****CAUTION**** 2,4-TDA, 2,6-TDA, 1,6-HDA and 4,4'-MDA are potential carcinogens so wear gloves while handling.

- 4.1.1.1 Weigh 10 mg of each of the diamines in 10 mL volumetric flasks and q.s. with 0.1 M H₂SO₄.

* 4.1.2 Diamine working standards (100 ug/mL)

- 4.1.2.1 With a volumetric pipette, aspirate 1 mL of each of the 1 mg/mL stock standards and dispense

in 10 mL volumetric flasks, q.s.
with 0.1 M H₂SO₄.

* 4.1.3 Diamine working standards (10 ug/mL)

4.1.3.1 With a volumetric pipette,
aspirate 1 mL of each of the 100
ug/mL working standards and place
in 10 mL volumetric flasks. Q.S.
with 0.1 M H₂SO₄.

* 4.1.4 Diamine working standards (1 ug/mL)

4.1.4.1 With a volumetric pipette,
aspirate 1 mL of each of the 10
ug/mL working standards and place
in 10 mL volumetric flasks, then
q.s. with 0.1 M H₂SO₄.

* 4.1.5 Diamine working standards (100 ng/mL)

4.1.5.1 With a volumetric pipette,
aspirate 1 mL of each of the 1
ug/mL working standards and place
in 10 mL volumetric flasks. Q.S.
with 0.1 M H₂SO₄

* Stable for 3 months if kept at 4°.

4.1.6 Saturated sodium hydroxide.

4.1.6.1 Weigh 77 mg of sodium hydroxide in
a 100 mL volumetric flask and q.s
with distilled deionized water.

4.1.6.2 Mix thoroughly. Keep at room
temperature.

4.1.7 1 M phosphate buffer, pH 7.0.

4.1.7.1 Weigh 156 g of sodium dihydrogen
orthophosphate and 142 g of
di-sodium hydrogen orthophosphate.

4.1.7.2 Add to a 1 liter volumetric flask
and q.s. with distilled deionized
water.

4.1.7.3 Adjust pH to 7.0

4.2 SUPPLIES

- 4.2.1 13 X 100 mm screw-cap centrifuge tubes with teflon-lined caps.
- 4.2.2 16 X 125 mm screw-cap centrifuge tubes with teflon-lined caps.
- 4.2.3 2,4-TDA 98% standard (Aldrich Chemical)
- 4.2.4 2,6-TDA 97% standard (Aldrich Chemical)
- 4.2.5 1,6-HDA standard (Sigma Chem. Co.)
- 4.2.6 4,4'-MDA 99% standard (Aldrich Chemical)
- 4.2.7 6 M HCl solution (BDH)
- 4.2.8 Sodium chloride salt (BDH)
- 4.2.9 Toluene (BDH)
- 4.2.10 Ethyl Acetate, distilled (BDH)
- 4.2.11 Heptafluorobutyryl anhydride (HFBA), (Sigma Chem. Co.)
- 4.2.12 Vials, 0.3 mL conical with seals, Canlab
- 4.2.13 Aluminum seals, 11 mm with PFTBE septa, Canlab
- 4.2.14 DB-1 polymethyl siloxane column 20 M X 0.25 mm i.d., film thickness 0.25µm, J&W Scientific

4.3 EQUIPMENT

- 4.3.1 Pierce Reacti-Therm heating module
- 4.3.2 Eberbach mechanical shaker
- 4.3.3 Hewlett-Packard 5890 Gas Chromatograph equipped with a HP 5970 Mass Spectral Detector, and a HP 7673A Automatic Liquid Sampler, were used for analysis.

5.0 CALIBRATION

5.1 Prepare the calibration standards as follows:

- 5.1.1 Using a Hamilton syringe add the following amounts of diamine to the appropriately labelled centrifuge tubes.

Concentration in ng/mL	Amount in uL
0	0 (100 ng/mL std)
0.1	2 "
0.5	10 "
1.0	20 "
5.0	10 (1 ug/mL std)
10.0	20

5.1.2 Add 2 mL blank urine to each tube, and 50 ng/mL of internal standard 4,4'-MDA (10 uL of 10 ug/mL).

5.1.3 Process standard samples according to sample extraction procedure.

5.1.4 A standard curve is prepared using the area ratio calculations. A slope and correlation are obtained.

6.0 QUALITY CONTROL

6.1 An in-house urine quality control is prepared.

6.2 The quality control is processed with the other samples to be analyzed.

6.3 The concentration is based on the area ratio calculations.

7.0 PROCEDURE

7.1 FREE EXTRACTION

7.1.1 Aliquot 2 mL of patient's and blank urine in 16 x 125 mm screw-cap centrifuge tubes. Add proper amount of standards to the blank urine to give concentrations of 0, 0.1, 0.5, 1.0, and 5.0 ng/mL.

7.1.2 Add 1.0 mL of saturated sodium hydroxide, 0.5 g of sodium chloride, 5 mL of toluene, and 50 ng/mL of internal standard 4,4'-MDA.

7.1.3 Shake on the Eberbach shaker for 10 minutes and centrifuge for 5 minutes at 1500 g.

7.1.4 Remove the toluene layer to another set of centrifuge tubes 13 x 100 mm.

7.2 HYDROLYSIS

7.2.1 A standard curve is obtained by adding the proper amount of standards to give final concentrations of 0, 0.5, 1, 5, and 10

ng/mL, to 2 mL of blank urine.

- 7.2.2 To 2 mL of patient's and standards, add 1 mL of 6 M HCl and 50 ng/mL of 4,4'-MDA (10 uL of 10 ug/mL).
- 7.2.3 Hydrolyze for 1 hour at 105°C.
- 7.2.4 Let cool to room temperature, and proceed as for the free extraction.

7.3 DERIVATIZATION

- 7.3.1 100 uL of heptafluorobutyryl anhydride (HFBA) is added to the organic phase and vortexed at high speed for 20 sec.
- 7.3.2 The mixture is left at room temperature for 10 minutes.
- 7.3.3 The excess derivatizing reagent is removed by extraction with 1 mL of 1 M phosphate buffer (pH 7.0.)
- 7.3.4 The toluene layer containing the amine derivatives is dried under a stream of nitrogen at 40°C.
- 7.3.5 The residue is redissolved in 100 uL of ethyl acetate and transferred to a 0.3 mL conical ALS vial.

7.4 ANALYSIS

- 7.4.1 2 uL of the residue is injected onto the GC/MSD system using method file DATA:AMNTDA.M
- 7.4.2 GC/MSD and ALS conditions are listed in Table 1.
- 7.4.3 Acquisition ions and retention time data are listed in Table 2.
- 7.4.4 Calculate the unknown and the standards' ion ratios.
- 7.4.5 The ion abundance ratios of the three ions programmed to identify the substance in the unknown sample MUST calculate within +/-20% of the ion abundance ratios of the same

three ions identifying the drug standards.

7.4.6 The ion abundance ratios of the two ions programmed to identify the internal standard in the unknown sample MUST calculate within +/-20% of the ion abundance ratios of the same two ions identifying the internal standard in the chosen standard.

7.4.7 If the above criterion of a suspected positive are not met the sample is reported as negative.

7.5 REPORTING

7.5.1 Results are reported in ug/mmol of creatinine by dividing the concentration of diamines by the creatinine concentration.

8.0 PROCEDURE ASSESSEMENT

8.1 Linearity

8.1.1 The calibration curves are linear to at least 1000ng/mL, with points at 0, 50, 100, 500, 1000 ng/mL.

8.2 Recovery

8.2.1 The recoveries were for 2,4 and 2,6-TDA's 96% and 92% respectively, for 1,6-HDA 111%, and 4,4'-MDA 103%.

8.3 Sensitivity

8.3.1 The detection limits in the GC-SIM mode are for 2,4 and 2,6-TDA's 100 pg/mL, for 1,6-HDA 1 ng/mL and 4,4'-MDA 2 ng/mL.

Table 1. Gas Chromatographic conditions for the analysis of diamines, 2,4 & 2,6-TDA, 1,6-HDA and 4,4'-MDA.

Column: DB-1 polymethyl siloxane, 20 m x 0.25 mm i.d.,
film thickness: 0.25 μ m

Carrier gas: Helium, linear velocity 30.5 cm/sec

Injector Mode : Splitless, purge on at 0.5 min.

Oven Temperature:

Initial:	115°C	Time 0.00 min
Rate:	20°C/min	
Final:	300°C	Time 2.00 min

Run Time: 15.5 min

Other temperatures:

Injection Port: 250°C
Transfer Line : 300°C

Equilibration Time: 0.00 min

Mass Spectral Detector Conditions:

Solvent delay: 5 min

Electron Multiplier: 200 volts relative

Source temperature: 200°

Configuration File: *ATUNE.U

Automatic Liquid Sampler Parameters:

Number of bottle A washes: 10 (distilled ethyl acetate

Number of bottle B washes: 10 (distilled ethyl acetate)

Number of sample washes: 0

Number of sample pumps: 3

Injector stroke length: 2

Sample Viscosity: 1

Injection Mechanism: Auto liquid sampler

Injection Mode: Fast

Table 2. Ions selected and retention times for the screening of the diamines

COMPOUND	RT (MIN)	IONS MONITORED DWELL TIME (MS)			GROUP	SCREEN WINDOW(MIN)
2,4-TDA	7.5	317 (50)	345 (50)	514 (50)	1	7.0-8.0
2,6-TDA	7.3	317 (50)	345 (50)	514 (50)	1	7.0-8.0
1,6-HDA	7.1	226 (50)	339 (50)	282 (50)	1	7.0:7.5
4,4'-MDA	14.1	132 (50)	302 (50)	590 (50)	2	13.5:14.5

9.0 REFERENCES

1. NIOSH Publications, CIB 53 (1989).
2. C. Rosenberg and H. Savolainen, J. Chromatogr 323 (1985) 429.
3. T. Brorson, G. Skarping, J.F. Sandstrom, and M. Stenberg, Int Arch Occup Environ Health 62, (1990) 79.
4. J. Cocker, L.C. Brown, and H.K. Wilson, J Anal Toxicol 12 (1988) 9.
5. I.B. Glowinski, W.W. Weber, J Biol Chem 257 (1982) 1424.

NLC - BNC



3 3286 11371478 2